BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

Search09813341

Set	Items	Description
S1	4300	TETRAVALENT
S2	24	TETRA(W)VALENT
S3	261	S1 AND ANTIBODY
S4	2150	DR5
S5	0	S3 AND S4
S6	13	S3 AND FC
S7	8	RD (unique items)

SYSTEM:OS - DIALOG OneSearch
File 155:MEDLINE(R) 1966-2003/Mar W2
(c) format only 2003 The Dialog Corp.
File 5:Biosis Previews(R) 1969-2003/Mar W1
(c) 2003 BIOSIS
***File 5: Alert feature enhanced for multiple files, duplicates** removal, customized scheduling. See HELP ALERT.
File 399:CA SEARCH(R) 1967-2003/UD=13811
(c) 2003 American Chemical Society
***File 399: Use is subject to the terms of your user/customer agreement.**
Alert feature enhanced for multiple files, etc. See HELP ALERT.

7/9/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

10473842 20007365 PMID: 10541352

Generation and characterization of a single gene-encoded single-chain- tetravalent **antitumor** antibody .

Santos A D; Kashmiri S V; Hand P H; Schlom J; Padlan E A

Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland 20892, USA.

Clinical cancer research : an official journal of the American Association for Cancer Research (UNITED STATES) Oct 1999, 5 (10 Suppl) p3118s-3123s, ISSN 1078-0432 Journal Code: 9502500

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Monoclonal **antibody** (mAb) CC49, a murine IgG1, reacts with the tumor-associated glycoprotein-72 expressed on a variety of carcinomas. In clinical trials, radiolabeled CC49 has shown excellent tumor localization to a variety of carcinomas. To minimize the immunogenicity of CC49 mAb in patients, a humanized CC49 (HuCC49) was generated by complementarity-determining region (CDR) grafting. The relative affinity of HuCC49 was 2-3-fold less than that of the murine mAb. With the aim of improving tumor targeting, attempts have been made to enhance the avidity of the HuCC49 mAb. Previous research has yielded a single gene-encoded immunoglobulin, SCIgcCC49deltaCH1, which is a dimer of a single chain consisting of CC49 single-chain Fv linked to the NH2 terminus of the human gamma1 **Fc** through the hinge region. This molecule is comparable to the mouse-human chimeric CC49 in terms of in vitro antigen binding properties, cytolytic activity, and rate of plasma clearance in athymic mice bearing human tumor xenografts. Recently, a single gene encoding a single-chain immunoglobulin consisting of a HuCC49 diabody attached to human gamma1 **Fc** via the hinge region was constructed. The diabody, a bivalent antigen-binding structure, is made up of variable heavy (V_H(H))/variable light (V(L)) domains and V(L)/V(H) domains. In each of the variable domain pairs, the V(H) and V(L) domains are linked through a short linker peptide. Meanwhile, the two pairs are linked via a 30-residue Gly-Ser linker peptide to yield two antigen-binding sites by lateral and noncovalent association of the V(L) of one pair with the V(H) of the other. Transfectomas expressing the single-gene immunoglobulin secrete a homodimer of about Mr 160,000 that reacts to tumor-associated glycoprotein-72. This **tetravalent** humanized antitumor immunoglobulin molecule may potentially be an efficacious therapeutic and diagnostic reagent against a wide range of human carcinomas.

7/9/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

10340764 99339447 PMID: 10413102

Novel tetravalent **and bispecific IgG-like** antibody **molecules combining single-chain diabodies with the immunoglobulin gamma1** Fc **or CH3 region.**

Alt M; Muller R; Kontermann R E

Institut fur Molekularbiologie und Tumorforschung, Philipps-Universitat Marburg, Germany.

FEBS letters (NETHERLANDS) Jul 2 1999, 454 (1-2) p90-4, ISSN

0014-5793 Journal Code: 0155157
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Although bispecific IgG molecules have been successfully applied for **antibody** -mediated immunotherapy of tumours, applicability is hampered by the difficulties associated with their generation. In the present study, we have used a bispecific single-chain diabody (scDb) directed against carcinoembryonic antigen and Escherichia coli beta-galactosidase as a model to generate bispecific IgG-like **antibody** molecules. We show that the fusion of this single-chain diabody to the **Fc** (scDb- **Fc**) or CH3 (scDb-CH3) region of the human immunoglobulin gamma1 chain results in the expression of dimeric fusion proteins exhibiting four functional antigen binding sites with increased functional affinity. This strategy represents a new and convenient way to generate IgG-like multivalent and bispecific molecules that are efficiently secreted from mammalian cells.

7/9/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09292454 97187689 PMID: 9035142
Design and production of novel tetravalent **bispecific antibodies.**
Coloma M J; Morrison S L
Department of Microbiology and Molecular Genetics, University of California at Los Angeles 90095, USA.
Nature biotechnology (UNITED STATES) Feb 1997, 15 (2) p159-63, ISSN 1087-0156 Journal Code: 9604648
Comment in Nat Biotechnol. 1997 Feb;15(2) 125-6; Comment in PMID 9035132
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

We have produced novel bispecific antibodies by fusing the DNA encoding a single chain **antibody** (ScFv) after the C terminus (CH3-ScFv) or after the hinge (Hinge-ScFv) with an **antibody** of a different specificity. The fusion protein is expressed by gene transfection in the context of a murine variable region. Transfectomas secrete a homogeneous population of the recombinant **antibody** with two different specificities, one at the N terminus (anti-dextran) and one at the C terminus (anti-dansyl). The CH3-ScFv **antibody** , which maintains the constant region of human IgG3, has some of the associated effector functions such as long half-life and **Fc** receptor binding. The Hinge-ScFv **antibody** which lacks the CH2 and CH3 domains has no known effector functions.

Searches for 09/813,341

SYSTEM:OS - DIALOG OneSearch
File 155:MEDLINE(R) 1966-2004/Feb W1
(c) format only 2004 The Dialog Corp.
***File 155: Medline is updating again (12-22-2003).**
Please see HELP NEWS 154, for details.
File 5:Biosis Previews(R) 1969-2004/Feb W1
(c) 2004 BIOSIS
File 159:Cancerlit 1975-2002/Oct
(c) format only 2002 Dialog Corporation
***File 159: Cancerlit ceases updating with immediate effect.**
Please see HELP NEWS.

Set	Items	Description
S1	1378	GLY(W)SER(W)GLY(W)SER
S2	49	S1 AND LINKER?
S3	16	S2 NOT PY=>1999
S4	7	RD (unique items)

4/9/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2004 The Dialog Corp. All rts. reserv.

11317445 98196936 PMID: 9537586
Construction and characterization of a bispecific diabody for retargeting T cells to human carcinomas.
Helfrich W; Kroesen B J; Roovers R C; Westers L; Molema G; Hoogenboom H R ; de Leij L
GUIDE, University Hospital, Department of Clinical Immunology, Groningen, The Netherlands.
International journal of cancer. Journal international du cancer (UNITED STATES) Apr 13 1998, 76 (2) p232-9, ISSN 0020-7136 Journal Code: 0042124
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
We describe the construction of a recombinant bispecific antibody fragment in the diabody format with specificity for both the well-established human pancarcinoma associated target antigen EGP2 (epithelial glycoprotein 2, also known as the C017-1A antigen or KSA) and the CD3epsilon chain of human TCR/CD3 complex. The murine anti-EGP2 (MOC31) single chain variable fragment (scFv) and the humanized anti-CD3 (Ucht1v9) scFv were cast into a diabody format (designated Dia5v9) using a short 5 amino acid **Gly - Ser linker** between immunoglobulin heavy-chain and light-chain variable domains. Purification of the poly-histidine tagged Dia5v9 was achieved from extracts of protease deficient Escherichia coli by IMAC chromatography. The Dia5v9 diabody showed strong binding to both EGP2 and CD3 in transfected cells. The in vitro efficacy of Dia5v9 in mediating tumor cell lysis by interleukin-2 activated human T cells appeared to be similar to that of the hybrid-hybridoma-derived BsF(ab')2 Bis1 (anti-EGP2/anti-CD3) in a standard 4-hr 51Cr-release assay. This small and partially humanized recombinant bispecific antibody fragment may be valuable for T-cell-based immunotherapeutical treatment protocols, retargeting activated peripheral blood T lymphocytes to lyse various human carcinomas in vivo.

4/9/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2004 The Dialog Corp. All rts. reserv.

08661606 95350203 PMID: 7624362
A small bispecific antibody construct expressed as a functional single-chain molecule with high tumor cell cytotoxicity.
Mack M; Riethmuller G; Kufer P
Institut fur Immunologie, Munich, Germany.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Jul 18 1995, 92 (15) p7021-5, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Construction of a bispecific single-chain antibody derivative is described that consists of two different single-chain Fv fragments joined through a **Gly - Ser linker** . One specificity of the two Fv fragments is directed against the CD3 antigen of human T cells and the other is directed against the epithelial 17-1A antigen; the latter had been found in a clinical trial to be a suitable target for antibody therapy of minimal residual colorectal cancer. The construct could be expressed in CHO cells as a fully functional protein, while its periplasmic expression in Escherichia coli resulted in a nonfunctional protein only. The antigen-binding properties of the bispecific single-chain antibody are indistinguishable from those of the corresponding univalent single-chain Fv fragments. By redirecting human peripheral T lymphocytes against 17-1A-positive tumor cells, the bispecific antibody proved to be highly cytotoxic at nanomolar concentrations as demonstrated by 51Cr release assay on various cell lines. The described bispecific construct has a molecular mass of 60 kDa and can be easily purified by its C-terminal histidine tail on a Ni-NTA chromatography column. As bispecific antibodies have already been shown to be effective in vivo in experimental tumor systems as well as in phase-one clinical trials, the small CD3/17-1A-bispecific antibody may be more efficacious than intact antibodies against minimal residual cancer cells.

***603612** Links

TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 10B; TNFRSF10B

Alternative titles; symbols

DEATH RECEPTOR 5; DR5
TNF-RELATED APOPTOSIS-INDUCING LIGAND RECEPTOR 2; TRAILR2
KILLER/DR5
TRICK2

Gene map locus 8p22-p21

TEXT

TRAIL, also called APO2L (603598), is a member of the tumor necrosis factor (TNF) family of cytokines and induces apoptosis in a wide variety of cells. Using ligand-based affinity purification and extracts of cell lines that undergo TRAIL-induced apoptosis, Walczak et al. (1997) identified a TRAIL receptor which they designated TRAILR2. Using a combination of PCR and library screening, the authors isolated cDNAs corresponding to the TRAILR2 coding region. The predicted 440-amino acid protein contains a putative signal peptide, 2 cysteine-rich pseudorepeats characteristic of TNF receptor (TNFR) family members, a transmembrane domain, and an intracellular death domain. The death domain of TRAILR2 shares 30% identity with that of TNFR1 (191190). Overall, the TRAIL receptors TRAILR2 and TRAILR1 (DR4; 603611) are 58% identical. As with TRAILR1, overexpression of TRAILR2 engaged a caspase-dependent apoptotic pathway. However, in contrast to TRAILR1, TRAILR2 mediated apoptosis via the intracellular adaptor molecule FADD (602457). Northern blot analysis indicated that TRAILR2 was expressed as a 4.4-kb mRNA in all tissues tested, with the highest levels of expression in peripheral blood lymphocytes, spleen, and ovary.

Independently, Screaton et al. (1997) and Wu et al. (1997) cloned cDNAs encoding TRAILR2, which they designated TRICK2 and KILLER/DR5 (death receptor-5), respectively. Screaton et al. (1997) determined that alternative pre-mRNA splicing generates 2 isoforms of TRICK2, which they referred to as TRICK2A and TRICK2B. The predicted 440-amino acid TRICK2B protein contains a 29-amino acid extension to the extracellular domain relative to TRICK2A. Wu et al. (1997) reported that

expression of the KILLER/DR5 gene was induced by DNA-damaging agents in a p53 (191170)-dependent manner. KILLER/DR5 was also induced by wildtype p53 overexpression in the absence of DNA damage, and overexpression of KILLER/DR5 led to apoptotic death of cancer cells.

MacFarlane et al. (1997), Pan et al. (1997), Sheridan et al. (1997) and Schneider et al. (1997) isolated cDNAs encoding TRAILR2, also called DR5, and a decoy TRAIL receptor, TRAILR3 (603613). These authors concluded that there is complex regulation of TRAIL-mediated signals.

Formation of a complex between APO2L and its signaling receptors, DR4 and DR5, triggers apoptosis by inducing the oligomerization of intracellular death domains. Hymowitz et al. (1999) reported the crystal structure of the complex between APO2L and the ectodomain of DR5. The structure shows 3 elongated receptors snuggled into long crevices between pairs of monomers of the homotrimeric ligand. The interface is divided into 2 distinct patches, one near the bottom of the complex close to the receptor cell surface and the other near the top. Both patches contain residues that are critical for high-affinity binding. A comparison to the structure of the lymphotoxin receptor complex (see 600979) suggested general principles of binding and specificity for ligand recognition in the TNF receptor superfamily.

By analysis of radiation hybrid panels, Walczak et al. (1997) mapped the TRAILR2 gene to 8p22-p21. Wu et al. (1997) confirmed this assignment using fluorescence in situ hybridization. Marsters et al. (1997) found that the TRAIL receptors DR4 (TRAILR1) and DR5, and the TRAIL decoy receptors DCR1 (TRAILR3) and DCR2 (603614) are all located at 8p21, suggesting that these genes arose from recent gene duplication events.

The 8p21 region contains a number of putative tumor suppressor genes and is a frequent site of translocations in head and neck tumors. Pai et al. (1998) determined the genomic structure of KILLER/DR5 and performed sequence analysis of all 10 coding exons in 20 primary head and neck cancers with allelic loss of 8p. To screen for a subset of mutations localized to the functional cytoplasmic death domain, they sequenced this region in an additional 40 primary head and neck cancers. They found 2 alterations, including a 2-bp insertion at a minimal repeat site, introducing a premature stop codon and resulting in a truncated protein. This KILLER/DR5 mutation was also present in the germline of the affected patient, and the tumor did not have a p53 mutation by sequence analysis. Transfection studies in head and neck squamous cell carcinoma and colon and ovarian carcinoma cell lines revealed loss of growth suppressive function associated with the tumor-derived KILLER/DR5 truncation mutant. These observations provided the first evidence for mutation of a TRAIL death receptor gene in a human cancer, leading to loss of its apoptotic function. The second alteration identified by Pai et al. (1998) was a single T-to-C point mutation at residue 1109 that resulted in an amino acid change from val to ala. This mutation was not present in the germline; however, sequence analysis of p53 in this tumor revealed a point mutation of T to C in codon 242, resulting in a change from arg to cys.

ALLELIC VARIANTS

(selected examples)

.0001 SQUAMOUS CELL CARCINOMA, HEAD AND NECK [TNFRSF10B, 2-BP INS]

In a case of a head and neck squamous cell carcinoma (601400), Pai et al. (1998) found a 2-bp insertion in the TNFRSF10B gene at a minimal repeat site (ACAC) at residue 1065, which introduced a premature stop codon and resulted in a truncated protein. Sequence analysis of normal tissue from the patient showed that the truncating mutation was also present in the germline, and that the tumor did not have a p53 mutation. A significant impairment in the ability of the truncation mutant to suppress colony formation was observed when mutant cDNA was transfected into human colon and ovarian cancer cell lines. In the wildtype transfected cells, there was no observed colony survival; however, there was more than 50% colony growth in cells transfected with the tumor-derived mutant. Pai et al. (1998) suspected that the mutant retained partial function, because its overexpression in a background of cells containing the endogenous wildtype gene could further reduce the percentage of colony survival.

REFERENCES

1. Hymowitz, S. G.; Christinger, H. W.; Fuh, G.; Ultsch, M.; O'Connell, M.; Kelley, R. F.; Ashkenazi, A.; de Vos, A. M. :
Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5. *Molec. Cell* 4: 563-571, 1999.
PubMed ID : 10549288

2. MacFarlane, M.; Ahmad, M.; Srinivasula, S. M.; Fernandes-Alnemri, T.; Cohen, G. M.; Alnemri, E. S. :
Identification and molecular cloning of two novel receptors for the cytotoxic ligand TRAIL. *J. Biol. Chem.* 272: 25417-25420, 1997.
PubMed ID : 9325248

3. Marsters, S. A.; Sheridan, J. P.; Pitti, R. M.; Huang, A.; Skubatch, M.; Baldwin, D.; Yuan, J.; Gurney, A.; Goddard, A. D.; Godowski, P.; Ashkenazi, A. :
A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr. Biol.* 7: 1003-1006, 1997.
PubMed ID : 9382840

4. Pai, S. I.; Wu, G. S.; Ozoren, N.; Wu, L.; Jen, J.; Sidransky, D.; El-Deiry, W. S. :
Rare loss-of-function mutation of a death receptor gene in head and neck cancer. *Cancer Res.* 58: 3513-3518, 1998.
PubMed ID : 9721851

5. Pan, G.; Ni, J.; Wei, Y.-F.; Yu, G.; Gentz, R.; Dixit, V. M. :
An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science* 277: 815-818, 1997.
PubMed ID : 9242610

6. Schneider, P.; Bodmer, J.-L.; Thome, M.; Hofmann, K.; Holler, N.; Tschopp, J. :

Characterization of two receptors for TRAIL. *FEBS Lett.* 416: 329-334, 1997.
PubMed ID : 9373179

7. Screaton, G. R.; Mongkolsapaya, J.; Xu, X.-N.; Cowper, A. E.; McMichael, A. J.; Bell, J. I. :
TRICK2, a new alternatively spliced receptor that transduces the cytotoxic signal from TRAIL. *Curr. Biol.* 7: 693-696, 1997.
PubMed ID : 9285725

8. Sheridan, J. P.; Marsters, S. A.; Pitti, R. M.; Gurney, A.; Skubatch, M.; Baldwin, D.; Ramakrishnan, L.; Gray, C. L.; Baker, K.; Wood, W. I.; Goddard, A. D.; Godowski, P.; Ashkenazi, A. :
Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science* 277: 818-821, 1997.
PubMed ID : 9242611

9. Walczak, H.; Degli-Esposti, M. A.; Johnson, R. S.; Smolak, P. J.; Waugh, J. Y.; Boiani, N.; Timour, M. S.; Gerhart, M. J.; Schooley, K. A.; Smith, C. A.; Goodwin, R. G.; Rauch, C. T. :
TRAIL-R2: a novel apoptosis-mediating receptor for TRAIL. *EMBO J.* 16: 5386-5397, 1997.
PubMed ID : 9311998

10. Wu, G. S.; Burns, T. F.; McDonald, E. R., III; Jiang, W.; Meng, R.; Krantz, I. D.; Kao, G.; Gan, D.-D.; Zhou, J.-Y.; Muschel, R.; Hamilton, S. R.; Spinner, N. B.; Markowitz, S.; Wu, G.; El-Deiry, W. S. :
KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. *Nature Genet.* 17: 141-143, 1997.
PubMed ID : 9326928

CONTRIBUTORS

Stylianos E. Antonarakis - updated : 11/19/1999
Victor A. McKusick - updated : 4/12/1999

CREATION DATE

Rebekah S. Rasooly : 3/5/1999

EDIT HISTORY

carol : 11/21/2000
mgross : 11/19/1999
carol : 5/10/1999
carol : 4/14/1999
terry : 4/12/1999
mgross : 3/9/1999
mgross : 3/8/1999